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**13. ABSTRACT (Maximum 200 Words)**

Ovarian carcinomas have a poor prognosis, often associated with multifocal intraperitoneal dissemination accompanied by intense neovascularization. To examine tumor angiogenesis in the tumor microenvironment, we studied malignant ascites of patients with untreated ovarian carcinoma. We observed high numbers of plasmacytoid dendritic cells (PDC) and significant stromal derived factor (CXCL-12/SDF)-1 in their malignant ascites attracting PDC into the tumor environment. We now show that tumor associated PDC induced angiogenesis *in vivo*. By contrast, myeloid dendritic cells (MDC) were absent from malignant ascites. MDC derived *in vitro* suppressed angiogenesis *in vivo*. Thus, the tumor may attract PDC to augment angiogenesis, while excluding MDC to prevent angiogenesis inhibition. Although dendritic cells have long been known to affect tumor immunity, these data also implicate them in tumor neoangiogenesis.

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## INTRODUCTION

Dendritic cells (DC) prime naïve T cells, and thereby activate antigen-specific immunity. The two principal human DC subtypes are MDC and PDC[1]. MDC expressing tumor antigens have been used in human clinical trials to induce significant clinical responses against some tumors[2]. Although much work has focused on the relevance of DC to tumor immunity, there are no reports regarding how DC influence tumor angiogenesis. Ovarian carcinomas have a poor prognosis, often associated with multifocal intraperitoneal dissemination accompanied by intense neovascularization. Because immune factors are known to modulate blood vessel formation in some settings[3], we hypothesized that specific DC subsets might differentially affect tumor neovascularization.

## BODY

To test this hypothesis, we studied patients with ovarian epithelial carcinomas International Federation of Gynecology and Obstetrics stage III or IV. All patients gave written, informed consent. The study was approved by the local Institutional Review Board. No patients received prior specific cancer treatments.

To study the role of tumor associated PDC in angiogenesis, we purified tumor associated PDC from malignant ascites as we described[4-6]. After activated with CD40L[7] (200 ng/ml, Immunex, Seattle, WA), we tested the *in vivo* angiogenic effects of tumor associated PDC in Matrigel[8,9]. We observed significant neovascularization in Matrigel plugs bearing tumor PDC (**Fig 1a**). As positive controls, the angiogenic cytokines vascular endothelial growth factor (VEGF) and FGF also induced strong angiogenesis *in vivo* (**Fig 1a**). The percentage of microvessel surfaces correlated with hemoglobin (Hb) content in Matrigel (**Fig 1b**) [10]. Histological analysis showed vascular channel formation and tortuous neovessels in Matrigel plugs bearing tumor PDC (**Fig 1c**) or FGF (**Fig 1d**), but not PBS (**Fig 1e**). These data demonstrate that tumor PDC directly induced angiogenesis *in vivo*.

We further explored the potential angiogeneic mechanisms of tumor associated PDC. Strikingly, tumor associated PDC ($10^6$/ml cultured 24 hours without stimulation) spontaneously produced high levels of the angiogenic cytokines TNF-α (190 ± 89 pg/ml) and interleukin (IL)-8 (160 ± 112 pg/ml) (n = 6). CD40L activation further enhanced PDC TNF-α (1033 ± 329 pg/ml) and IL-8 (840 ± 239 pg/ml) production (n = 6). Tumor associated PDC produced undetectable VEGF and IL-12 (not shown). We hypothesized that tumor PDC-derived TNF-α or IL-8 mediated PDC-driven angiogenesis. Consistent with this hypothesis, anti-TNF-α or anti-IL-8 antibody significantly decreased PDC-mediated angiogenesis *in vivo* (**Fig 1f**). In confirmation, recombinant TNF-α and IL-8 induced significant angiogenesis *in vivo* (**Fig 1f**). Thus, tumor PDC-induce angiogenesis at least in part through TNF-α and IL-8 production *in vivo*.

We observed no significant numbers of MDC in malignant ascites in patients with ovarian cancers[5]. To evaluate the potential angiogeneic role of tumor associated MDC *in vivo*, we differentiated tumor associated MDC from malignant ascites macrophages as we described. Tumor associated MDC did not induce angiogenesis *in vivo,* even after activating with CD40L.

Recombinant human VEGF induced significant angiogenesis *in vivo* (**Fig 2a**). Interestingly, VEGF mediated-angiogenesis was significantly reduced by tumor associated MDC (**Fig 2a,b**), indicating that MDC suppress angiogenesis *in vivo*. Further, we detected high levels of VEGF by ELISA in malignant ascites (1440 ± 739 pg/ml, n = 12) and primary ovarian carcinoma tumor cell cultures (340 ± 139 pg/ml VEGF produced by $10^6$ tumor cells/ml in 48 hours). Thus, tumor associated MDC suppressed angiogenesis induced by tumor derived VEGF.

Interestingly, anti-IL-12 antibody blocked the suppressive effects of MDC on angiogenesis (**Fig 2**). In confirmation, we showed that after CD40L activation, tumor associated MDC ($10^6$/ml activated for 24 hours) produced significant IL-12 (1830 ± 250 pg/ml, n = 8). In support, recombinant IL-12 inhibited VEGF-mediated angiogenesis (**Fig 2**). The data suggest that MDC are able directly to suppress angiogenesis *in vivo* through IL-12 production.

There are substantial numbers of functional PDC, but not MDC in tumor ascites in patients with ovarian carcinomas[5]. MDC, rather than PDC, produce significant amounts of IL-12 and induce high level of T cell interferon (IFN)-γ[5]. IL-12 and IFN-γ are potent angiogenic inhibitory cytokines. We hypothesize that MDC may suppress tumor angiogenesis *in vivo* through these cytokines. Lack of MDC in the tumor microenvironment may be orchestrated by the tumor to minimize the angiogenesis-inhibiting effects of these MDC. Consistent with our hypothesis, we showed here that MDC significantly suppressed angiogenesis *in vivo*. MDC derived IL-12 is a critical factor inhibiting tumor angiogenesis. These data may help explain the beneficial effects of MDC to reduce tumor burden even when not bearing tumor-associated antigens.

We recently demonstrated that tumor PDC inhibit anti-tumor immunity[5]. We now show that tumor PDC produce high levels of the angiogenic cytokines TNF-α and IL-8, and induce potent neovascularization *in vivo*. Thus, tumor associated PDC may be critical for tumor angiogeneisis.

**Original figures are attached.**

**Fig 1. Tumor PDC induced angiogenesis *in vivo*.** NOD.SCID mice (6-8 weeks old; Jackson Laboratory, Bar Harbor, ME) were inoculated with Matrigel plugs (Becton-Dickinson, San Jose, CA) bearing activated tumor associated PDC ($10^6$/ml) with or without the indicated reagents. After 12 days, we quantified the microvessel density (MVD) and content of hemoglobulin (Hb) in Matrigel. (**a**) Matrigel plugs were subjected to immunohistochemistry with anti-vWF antibody (polyclonal, 1/10 dilution, DAKO, Carpinteria, CA). MVD was analyzed[11] with ImagePro Plus software (Image-Pro plus, Media Cybernetics, Silver Spring, MD), and expressed as mean percentage of microvessel surface area by confocal Leica TCS-NT SP microscope. (**b**) Hb content in Matrigel plugs was detected with a commercial kit (Sigma, St. Louis, MO). (**c-e**) Histological analysis showed vascular channel formation and tortuous neovessels in Matrigel plugs with (**c**) tumor associated PDC, (**d**) FGF and (**e**) PBS. Green, vWF; Red, Topro. (**f**) Tumor associated PDC-derived IL-8 and TNF-α induced angiogenesis *in vivo*. Recombinant human VEGF, FGF, TNF-α, IL-8 (all 10 ng/ml) were from R & D system (Minneapolis, MN). Mouse anti-human TNF-α, and mouse anti-human-IL-8 antibody (500 ng/ml each) were from BD PharMingen (San Diego, CA). 7-10 mice per group.

**Fig 2. Tumor MDC suppress angiogenesis *in vivo*.** NOD.SCID mice were inoculated with Matrigel plugs bearing activated tumor associated MDC ($10^6$/ml) plus indicated reagents. Day 12 Matrigel plugs were removed to study neovascularization as described in the legend to Fig. 1. (**a**). MDC suppress VEGF-induced angiogenesis through IL-12. (**b**). Microvessel surface data is correlated with the Hb contents in Matrigel plugs. Recombinant human VEGF (10 ng/ml; R & D system), and mouse anti-human IL-12 antibody (500 ng/ml) were from BD PharMingen. 7-10 mice per group.

## KEY RESEARCH ACCOMPLISHMENTS

Tumor associated PDC induced angiogenesis in vivo.

Tumor associated PDC IL-8 and TNF a are the critical angiogenesis factors

Tumor associated MDC suppressed angiogenesis in vivo

Tumor associated MDC derived IL-12 is critical for suppressing angiogenesis in vivo.

## REPORTABLE OUTCOMES

### 1. Published manuscripts:

**Zou W**, Wei S, Curiel T. "Dendritic cells" in Gene Therapy. D. Curiel and J. Douglas, eds. Blackwell publishing, in press.

Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, Mottram P, Krzysiek R, Knuston K, Daniel B, Zimmermann MC, David O, Burow M, Gordon A, Dhurandhar N, Myers L, Berggren R, Hemminki A, Alvarez RD, Emilie D, Curiel DT, Chen L, **Zou W**. Blockade of B7-H1 Improves Myeloid Dendritic Cell-Mediated Anti-Tumor Immunity. **Nat Med 2003, 9:562-567.**

### 2. Published abstract:

American association of immunologists, 90th Anniversary Annual Meeting, May 6-10, 2003, Denver, Abstract #161.5.

## CONCLUSIONS

In summary, our data demonstrate a novel role for DC in human cancer. The DC system is relevant to tumor angiogenesis: MDC inhibit, and PDC enhance tumor angiogenesis. Maximal vascularization of tumors may thus require the simultaneous accumulation of PDC and the absence of MDC, as observed in ovarian tumors. Blocking PDC trafficking or function in tumors may be a novel strategy to block tumor neoangiogenesis.

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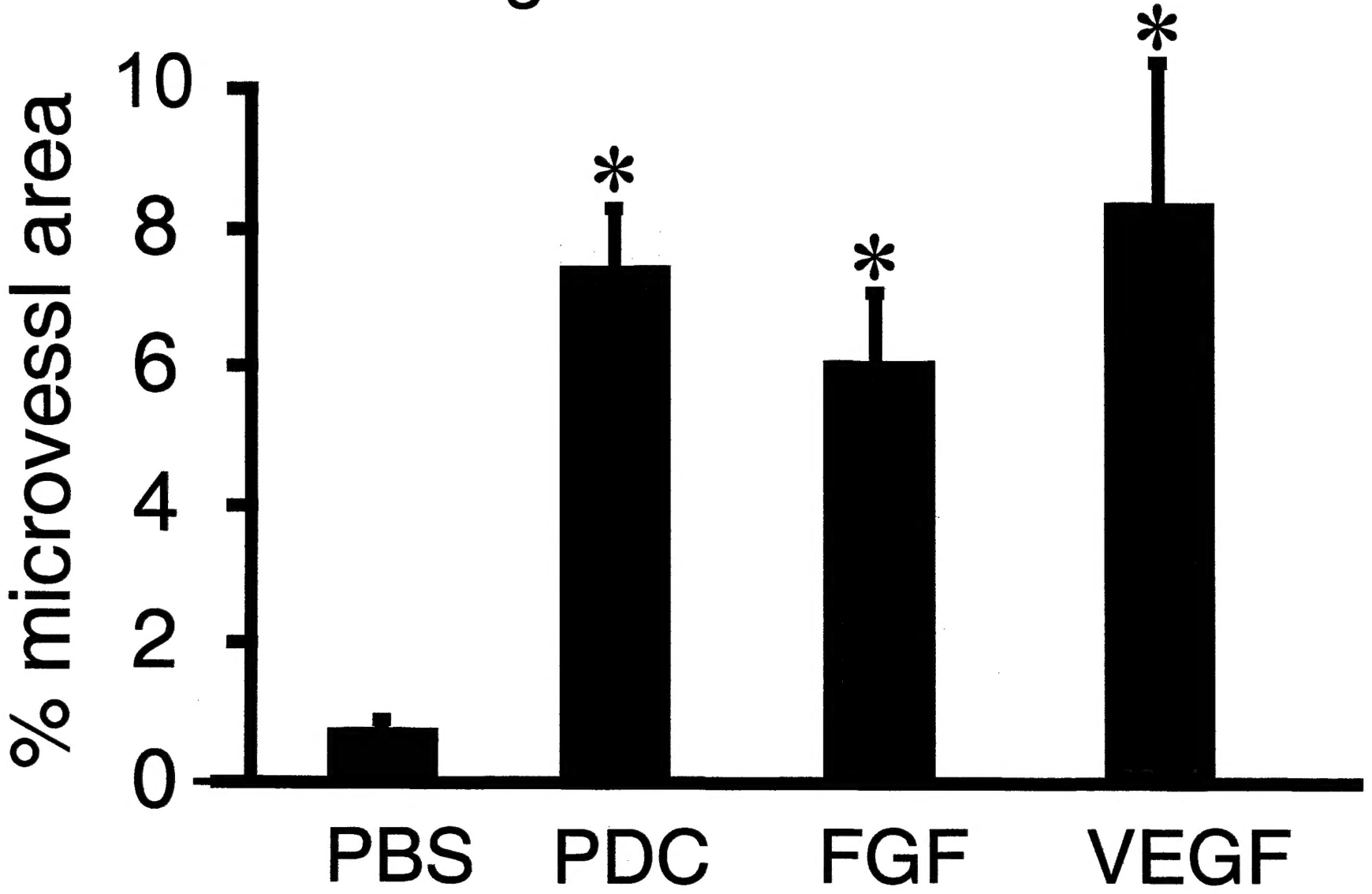
## APPENDICES

**Included** 1 Figure data set, 1 original copy of journal articles, 1 reprint of abstract, a curriculum vitae.

Fig 1a



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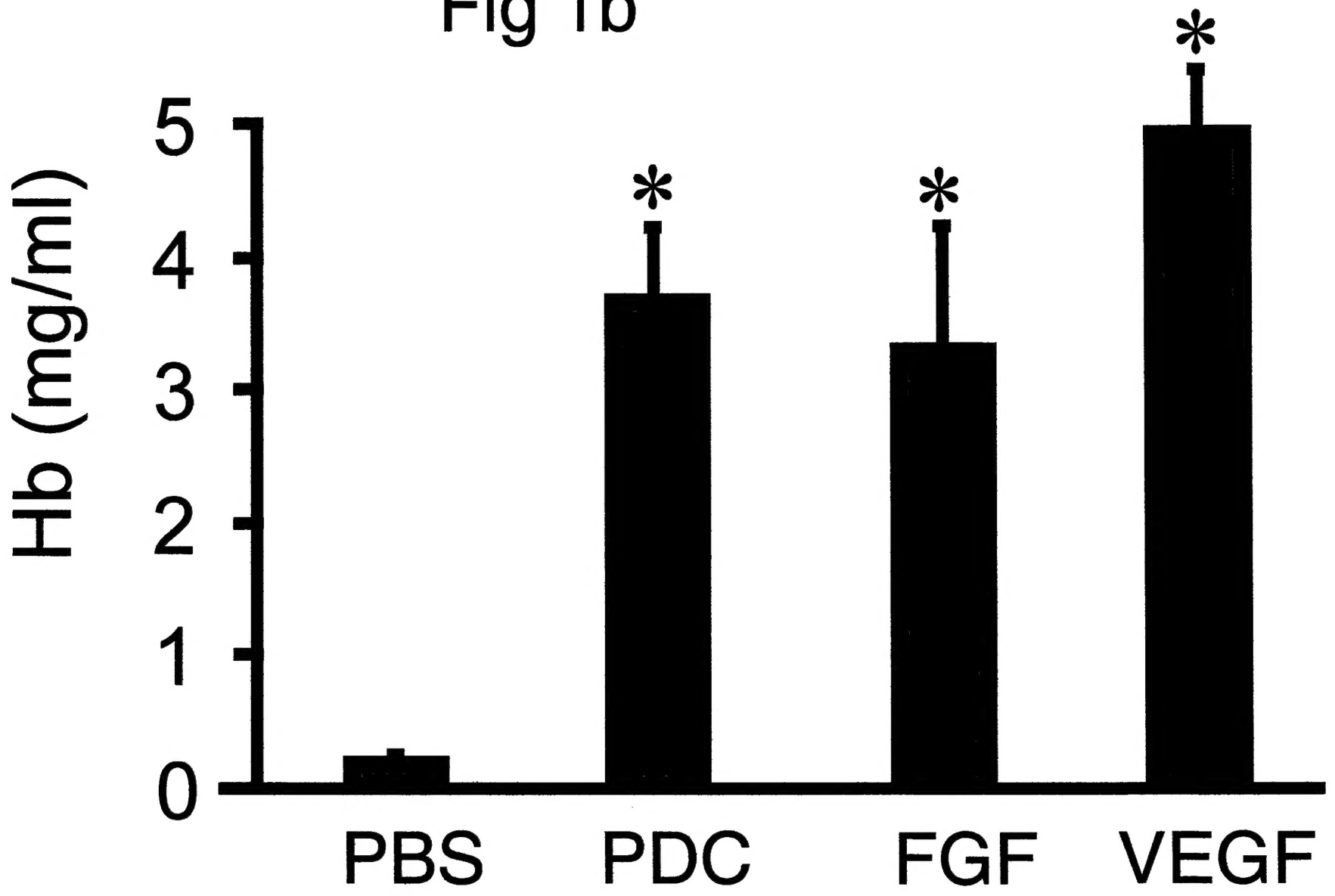

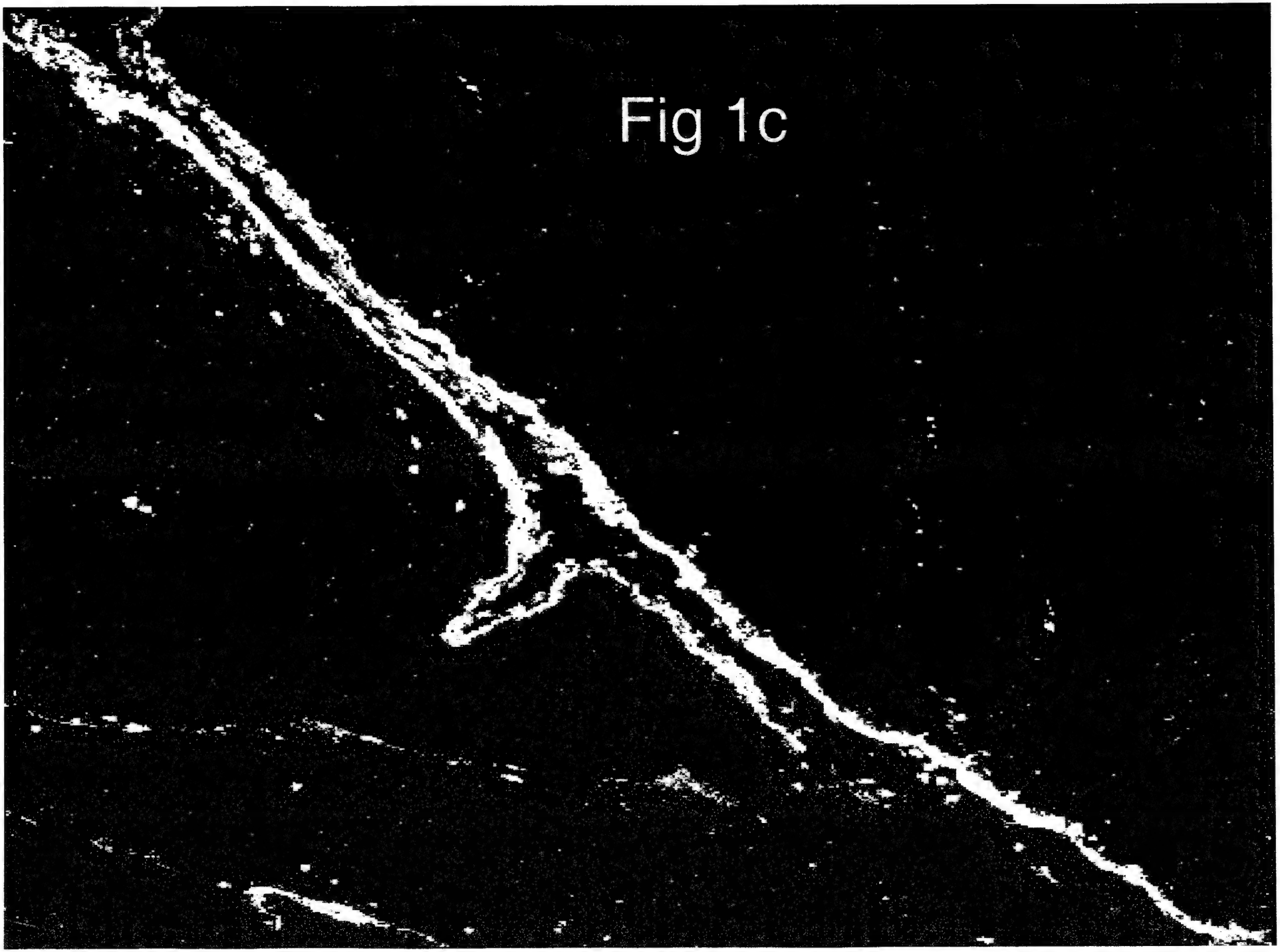
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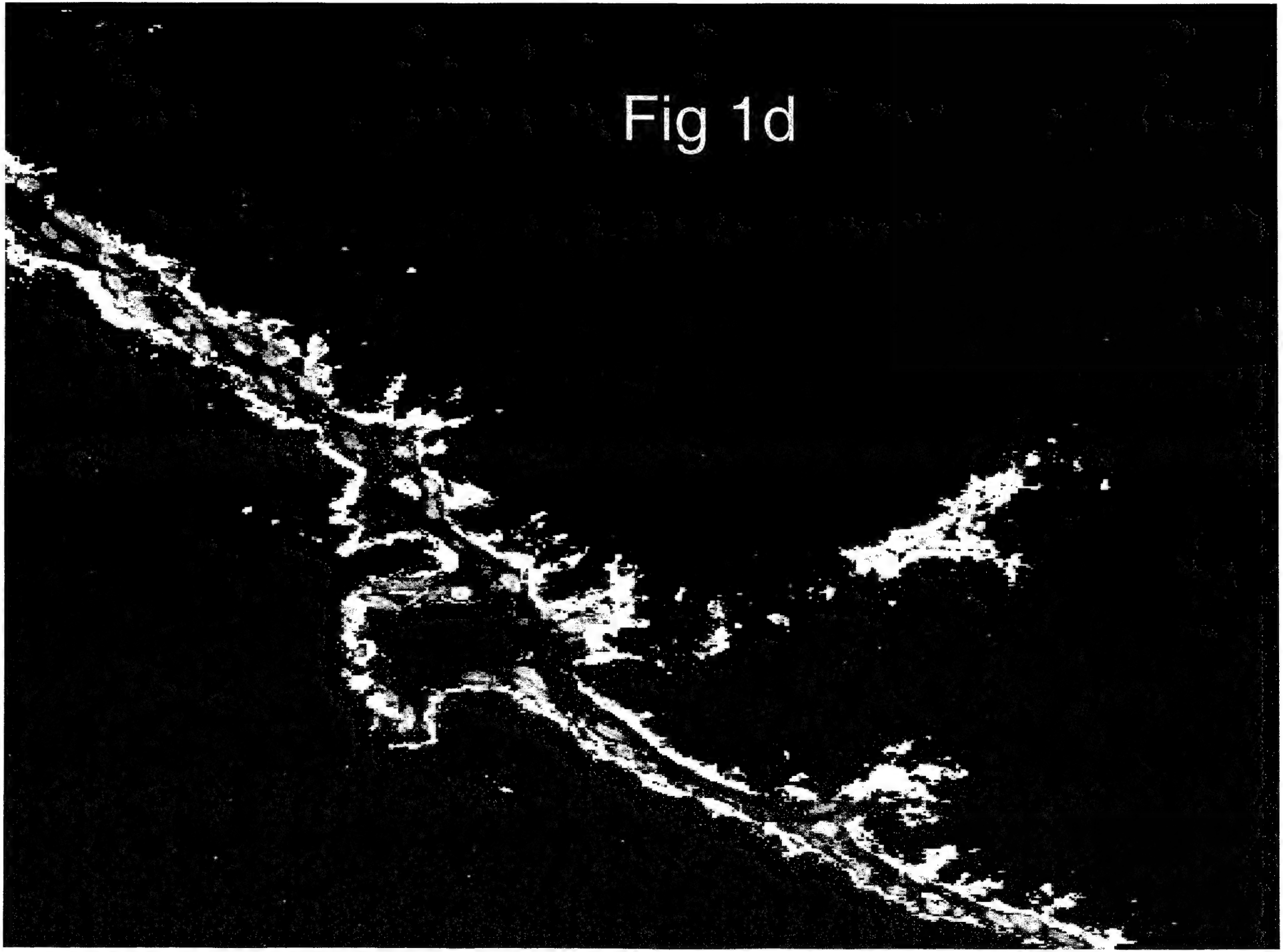
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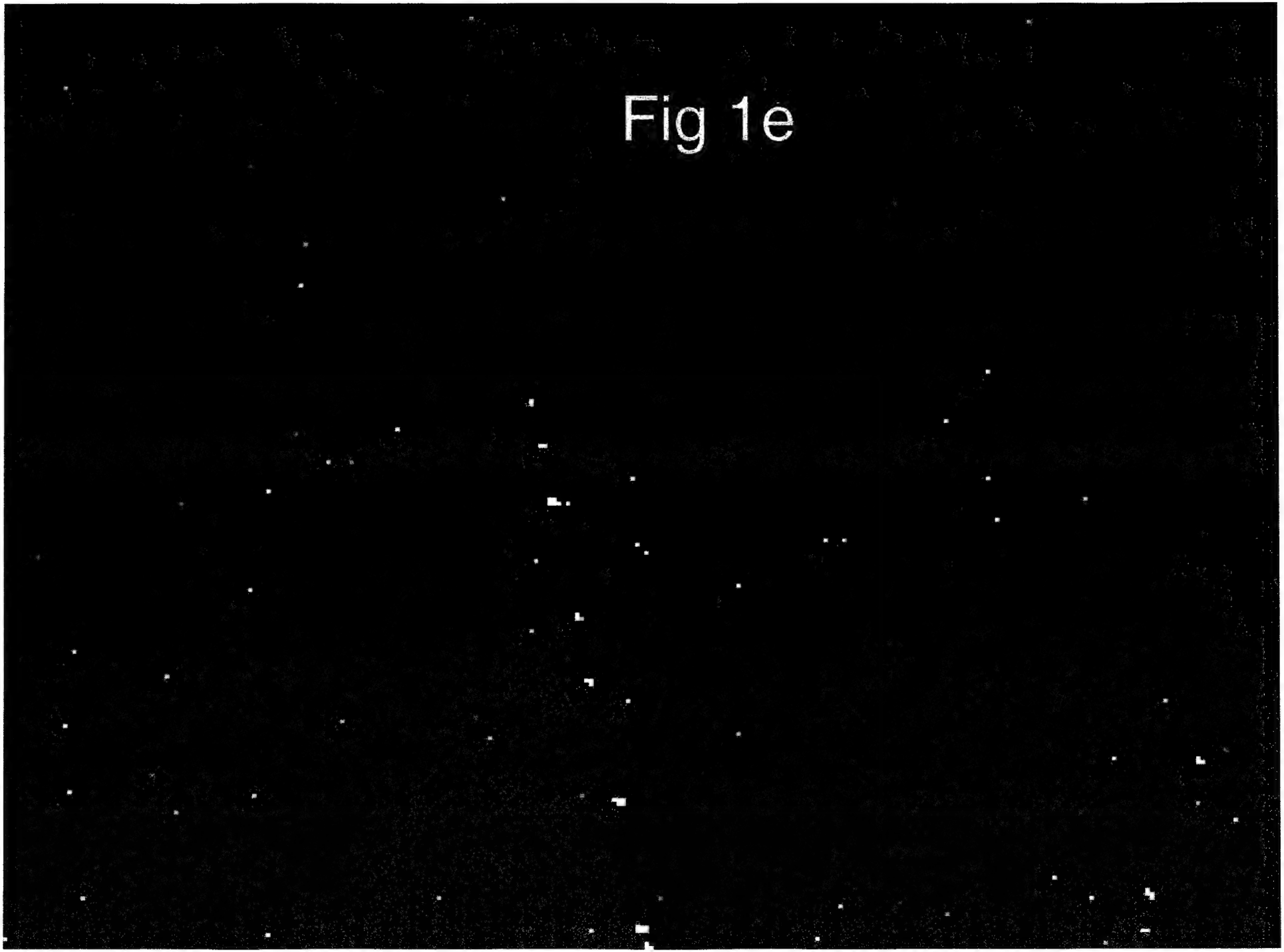
Fig 1e

Fig 2a

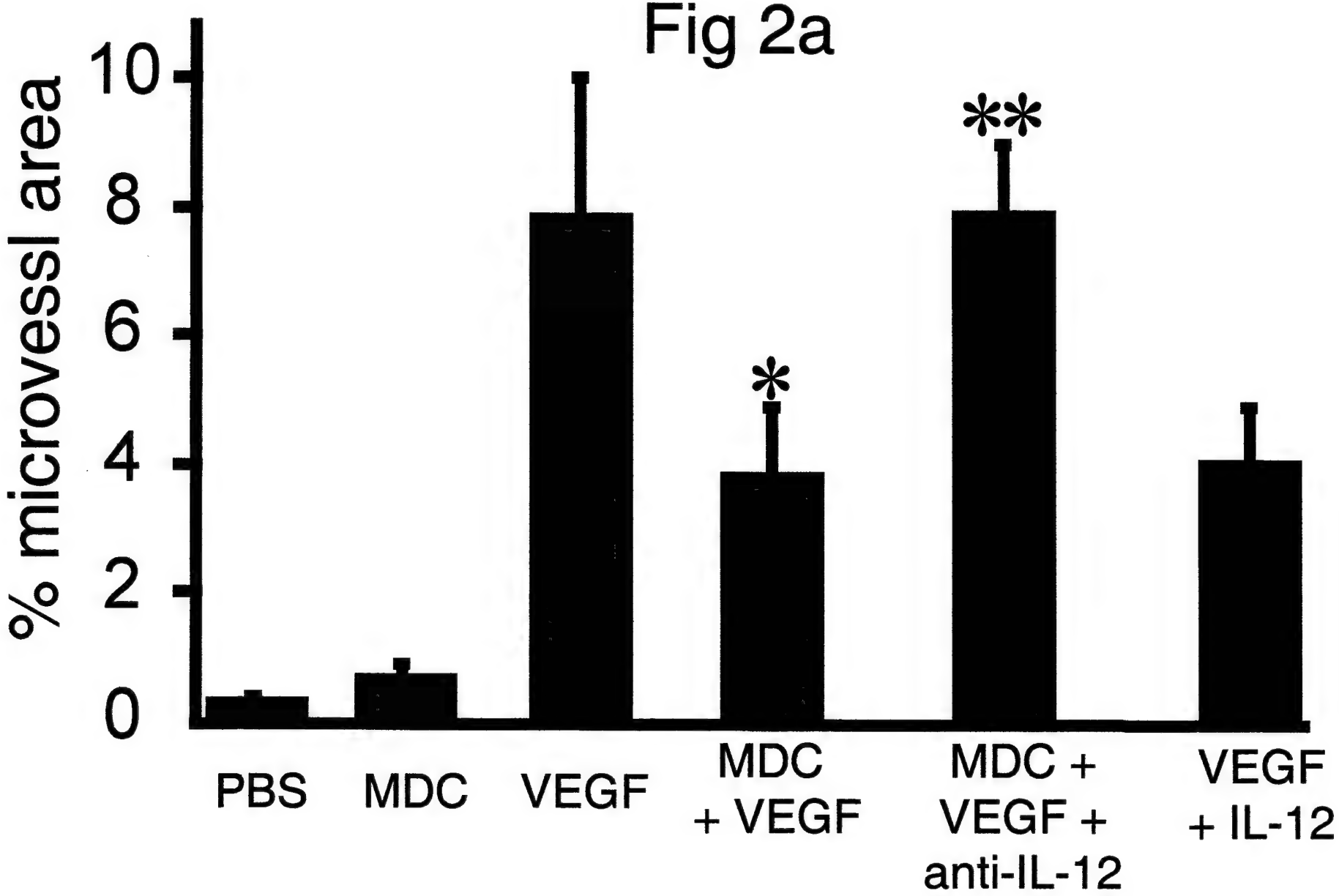
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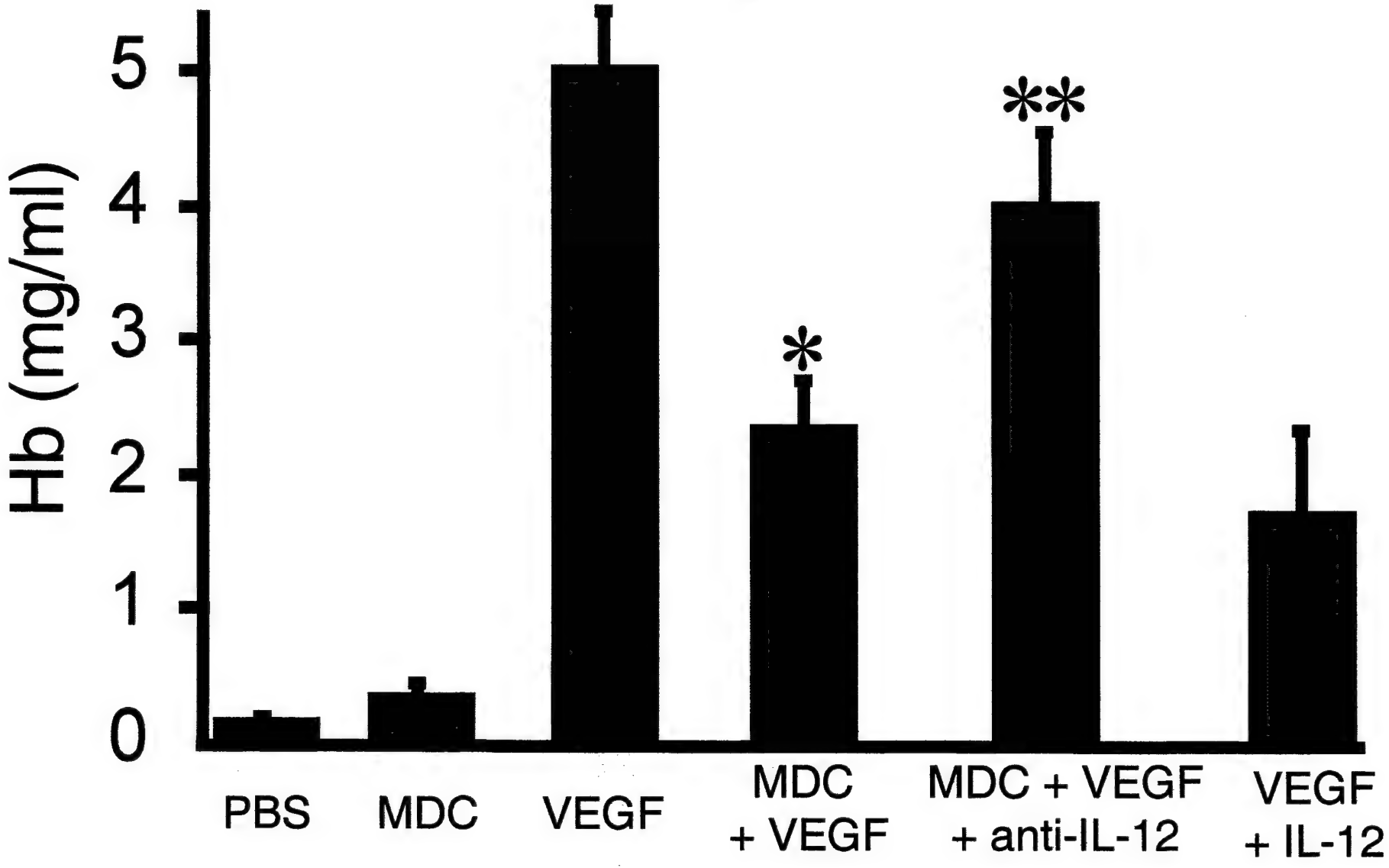
Fig 2b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VEGF + IL-12

# Blockade of B7-H1 improves myeloid dendritic cell–mediated antitumor immunity

TYLER J. CURIEL[1], SHUANG WEI[1], HAIDONG DONG[2], XAVIER ALVAREZ[1], PUI CHENG[1], PETER MOTTRAM[1], ROMAN KRZYSIEK[3], KEITH L. KNUTSON[4], BEN DANIEL[1], MARIA CARLA ZIMMERMANN[1], ODILE DAVID[1], MATTHEW BUROW[1], ALAN GORDON[5], NINA DHURANDHAR[1], LEANN MYERS[1], RUTH BERGGREN[1], AKSELI HEMMINKI[6], RONALD D. ALVAREZ[6], DOMINIQUE EMILIE[3], DAVID T. CURIEL[6], LIEPING CHEN[2] & WEIPING ZOU[1]

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**Suppression of dendritic cell function in cancer patients is thought to contribute to the inhibition of immune responses and disease progression. Molecular mechanisms of this suppression remain elusive, however. Here, we show that a fraction of blood monocyte-derived myeloid dendritic cells (MDCs) express B7-H1, a member of the B7 family, on the cell surface. B7-H1 could be further upregulated by tumor environmental factors. Consistent with this finding, virtually all MDCs isolated from the tissues or draining lymph nodes of ovarian carcinomas express B7-H1. Blockade of B7-H1 enhanced MDC-mediated T-cell activation and was accompanied by downregulation of T-cell interleukin (IL)-10 and upregulation of IL-2 and interferon (IFN)-γ. T cells conditioned with the B7-H1–blocked MDCs had a more potent ability to inhibit autologous human ovarian carcinoma growth in non-obese diabetic–severe combined immunodeficient (NOD-SCID) mice. Therefore, upregulation of B7-H1 on MDCs in the tumor microenvironment downregulates T-cell immunity. Blockade of B7-H1 represents one approach for cancer immunotherapy.**



MDCs have a pivotal role in adaptive immunity by activating naive T cells[1]. In animal models[2–4] and human clinical trials[5,6], adoptively transferred MDCs expressing tumor antigens mediate substantial antitumor immunity. Nevertheless, substantial protective immunity is rarely engendered naturally in tumor-bearing hosts[7]. Suppression of MDC differentiation and function in cancer patients may contribute to inhibition of immune responses and cancer progression[1,8,9].

B7-H1 (also called PD-L1) is a cell-surface glycoprotein belonging to the B7 family of costimulatory molecules. B7-H1 mRNA is found in a variety of non-lymphoid parenchymal organs, including the heart, placenta, skeletal muscle and lung, and B7-H1 protein has been detected in most human cancers, including ovarian cancers[10–12]. B7-H1 co-stimulates T-cell growth, selectively induces IL-10 during priming of T cells[10,11] and promotes programmed cell death of effector T cells through ligation of an unknown receptor[12]. In addition, B7-H1 is thought to inhibit T-cell growth by ligation of the PD-1 receptor[13], which is expressed on activated T and B cells[10,14]. PD-1 knockout mice develop spontaneous autoimmune diseases[15,16], suggesting a role for PD-1 in the inhibition of T-cell responses.

Many human cancers, including the majority of ovarian carcinomas, express B7-H1. Tumor-associated B7-H1 induces programmed cell death of effector T cells and is thought to contribute to immune evasion by cancers[12]. B7-H1 can be upregulated in various types of cells[12,17], however. Here, we show that B7-H1 is also upregulated on the surface of tumor-associated MDCs and may contribute to the suppressive functions of tumor-infiltrating MDCs in patients with previously untreated ovarian carcinomas. We undertook the present studies to determine the immunopathologic significance of this phenomenon.

### Expression of B7-H1 on MDC from ovarian cancer patients

To evaluate a potential role for MDC B7-H1 in tumor immunopathology, we evaluated MDCs in the lymph nodes draining the retroperitoneum of patients with ovarian carcinoma (tumor LN-MDC). We also generated MDCs from blood monocytes (blood MDCs) and tumor ascites $CD14^+$ cells (tumor MDCs). B7-H1 was weakly expressed on blood MDCs and MDCs from lymph nodes of tumor-negative subjects (control LN-MDCs). By contrast, the percentage of B7-H1–positive MDCs and the intensity of B7-H1 expression were signifi-

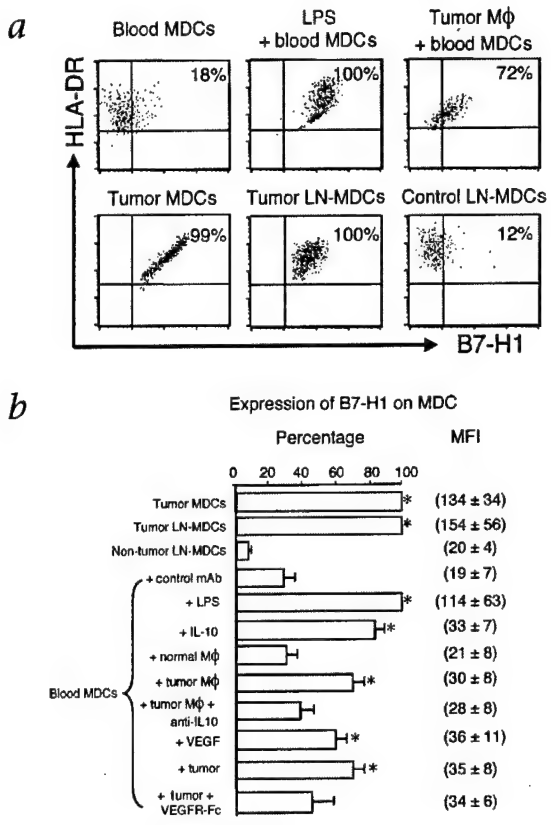



**Fig. 1** Expression and regulation of tumor MDC–associated B7-H1. ***a*** and ***b***, B7-H1 expression (*a*) and regulation (*b*) in tumor MDCs, blood MDCs and LN-MDCs were determined by FACS gated on $CD1a^+HLA\text{-}DR^{bright}$ cells for blood MDCs and tumor MDCs, and on $CD11c^+HLA\text{-}DR^{bright}$ cells for fresh LN-MDCs. Mean ± s.e.m. of the mean percentage of positive cells (*a*) and mean ± s.e.m. of the mean fluorescence intensity (MFI; *b*) are shown for 5 experiments with different blood MDCs and LN-MDCs. *, $P < 0.05$ as compared with blood MDCs with or without control monoclonal antibody (mAb). MΦ, macrophage; LPS, lipopolysaccharide; anti-IL10, antibody against IL-10.

cantly ($P < 0.05$) higher on both tumor LN-MDCs and tumor MDCs (Fig. 1*a* and *b*).

### Tumor environmental factors increase MDC B7-H1

To investigate microenvironmental factors contributing to MDC B7-H1 upregulation, we incubated immature blood MDCs with IL-10 and vascular endothelial growth factor (VEGF), as these cytokines are known to be in the ovarian carcinoma microenvironment. MDC B7-H1 expression was significantly increased by IL-10 and VEGF ($P < 0.05$; Fig. 1*b*). High concentrations of IL-10 (40 ng/ml) can moderately decrease dendritic cell CD86 expression[18]. VEGF (20–40 ng/ml) can suppress MDC maturation from $CD34^+$ progenitor cells[9]. We did not observe a significant effect of IL-10 (1–20 ng/ml) or VEGF (1–20 ng/ml) on blood MDC CD80 or CD86 expression (data not shown). Recombinant stromal-derived factor-1 (SDF-1; 200 ng/ml) had no significant effect on MDC B7-H1 expression.

We[8] and others[19] previously showed spontaneous secretion of IL-10 by tumor-associated macrophages. In the present study, we observed VEGF secretion from primary ovarian carcinoma cell lines (430 ± 126 pg/ml in supernatants; $n = 5$) and in malignant ascites (7,530 ± 2,567 pg/ml; $n = 6$), consistent with previous reports[20], which suggests that tumor macrophages and epithelial tumor cells contribute to upregulated tumor MDC B7-H1 expression. Consistent with this concept, incubation of blood MDCs with tumor macrophages, but not normal macrophages, significantly ($P < 0.05$) upregulated MDC B7-H1 expression, which was reversed by a neutralizing monoclonal antibody against IL-10. In addition, incubation of MDCs with primary ovarian cancer cells upregulated MDC B7-H1 expression, which was inhibited by blocking VEGF binding (Fig. 1*a* and *b*).

We further explored whether tumor environmental factors (VEGF and IL-10) could modulate B7-H1 expression on activated MDCs. After a 48-h exposure to lipopolysaccharide (1 μg/ml), 97% of blood MDCs expressed a mature phenotype ($CD1a^+HLA\text{-}DR^{bright}CD83^+$). Lipopolysaccharide activation significantly increased MDC B7-H1 expression (Fig. 1*a* and *b*). VEGF (20 ng/ml) and IL-10 (20 ng/ml), however, had no significant effects on lipopolysaccharide-matured MDC B7-H1, CD40, CD80 or CD86 expression (data not shown).

### Tumor factors impair MDC-mediated T-cell function

To explore the functional consequences of upregulated B7-H1 on MDC by tumor environmental factors, we exposed blood MDCs to tumor macrophages or IL-10 to upregulate B7-H1, and used these MDCs to stimulate allogeneic T cells. MDCs previously exposed to IL-10 or tumor macrophages, but not normal macrophages, were impaired in their capacity to stimulate T-cell proliferation. Addition of a monoclonal antibody against B7-H1 completely recovered the impaired T-cell proliferation (Fig. 2*a*). Thus, tumor microenvironmental factors, including macrophages and IL-10, impair MDC-mediated T-cell activation by upregulating B7-H1 expression on MDCs.

### Blocking B7-H1 increases MDC-mediated T-cell activation

To determine the role of MDC-associated B7-H1 in tumor immunity, we activated allogeneic human T cells with tumor MDCs, which induced significant T-cell IL-10 production ($P < 0.05$; Fig. 2*b* and *c*) from both $CD4^+$ and $CD8^+$ T cells (Fig. 2*c*, panel 4). Parallel to our prior observations regarding blood versus tumor plasmacytoid dendritic cells in ovarian carcinoma[8], T-cell IL-10 expression induced by tumor MDCs was significantly higher than that induced by blood MDCs (298 ± 45 compared with 134 ± 37 pg/ml, respectively; $P < 0.01$; $n = 4$). Blocking B7-H1 on tumor MDCs using a specific monoclonal antibody (clone 5H1) significantly increased T-cell IFN-γ and IL-2 production and decreased IL-10 production ($P < 0.05$; Fig. 2*b* and *c*).

The monoclonal antibody 5H1 does not block binding of B7-H1 to PD-1 in ELISA and fluorescence-activated cell sorting (FACS) analysis (data not shown). In addition, blocking the interaction between B7-H1 and PD-1 by soluble PD-1Ig had minimal effect on the production of T-cell IFN-γ, IL-10 and IL-2 (Fig. 2*b* and *c*). Soluble PD-1Ig efficiently bound B7-H1–transfected 293 cells (Fig. 2*d*); therefore, soluble PD1Ig is thought to block T-cell PD1 binding MDC B7-H1 (ref. 12). Thus, although PD-1 is the only identified receptor for B7-H1 (ref. 13), it is possible that the effect of MDC B7-H1 may be mediated by a non-PD-1 receptor on T cells.

Similar experiments were done with lipopolysaccharide-activated tumor MDCs. As expected, a monoclonal antibody against B7-H1 significantly increased T-cell IFN-γ (4,432 ± 732 using control antibody compared with 8,642 ± 889 pg/ml using anti-

B7-H1 monoclonal antibody; $P < 0.01$; $n = 4$), IL-2 (189 ± 68 using control antibody compared with 420 ± 112 pg/ml using anti-B7-H1 monoclonal antibody; $P < 0.01$) and decreased T-cell IL-10 (86 ± 22 using control antibody compared with 36 ± 25 pg/ml using anti-B7-H1 monoclonal antibody; $P < 0.01$) induced by activated MDCs, suggesting that the effect of B7-H1 is independent of the MDC activation and maturation status.

### B7-H1 blockade upregulates MDC IL-12 and downregulates IL-10

To determine the mechanism of B7-H1 blockade in modulation of T-cell responses, we examined the effects on MDCs of treatment with monoclonal antibody against B7-H1. Twenty-four hours after engaging T cells, MDC IL-12 p70 was detected by intracellular staining (10 ± 6% of cells positive) but not by ELISA of culture supernatants. After 48 h, 37% of MDCs expressed intracellular IL-12 p70 (Fig. 3*a*, panel 1), which was also detected in culture supernatants (Fig. 3*b*). Antibody against B7-H1 significantly ($P < 0.05$) increased expression of MDC IL-12 (Fig. 3*a*, panel 2 and *b*) but not the CC-chemokine macrophage inflammatory protein (MIP)-1α (data not shown). By contrast, 48 h after blocking B7-H1, MDC IL-10 production was significantly ($P<0.05$) lower ($P < 0.05$; Fig. 3*a*, panel 5 and *c*). Cytokine production was also confirmed by competitive RT-PCR of MDCs (Fig. 3*d*). Similarly, inclusion of PD-1Ig did not significantly increase MDC IL-12 (Fig. 3*a*, panel 3) or decrease MDC IL-10 expression (Fig. 3*a*, panel 6) compared with the monoclonal antibody against B7-H1 (Fig. 3*a*, panels 2 and 5). Our results indicate that blockade of B7-H1 modulates the cytokine production of MDCs, which may be responsible, at least in part, for enhancing T-cell responses.

Because tumor MDCs express large amounts of B7-H1, it is possible that the monoclonal antibody against B7-H1 directly affected MDC activation or function. To address this possibility, we included the monoclonal antibody against B7-H1 in the culture of MDCs alone, in the absence of T cells. The monoclonal antibody against B7-H1 did not induce any detectable effects on MDC phenotype (CD40, CD54, CD80, CD86 or HLA-DR expression) or cytokine or chemokine production (IL-12, IL-10 or MIP-1α) at up to 48 h with or without lipopolysaccharide-mediated activation (data not shown). In addition, MDCs did not express PD-1, as assessed by FACS analysis using a specific monoclonal antibody (data not shown). We also excluded a direct role of soluble monoclonal antibody against B7-H1 in activating T cells because inclusion of the antibody did not stimulate IL-2 or IFN-γ secretion from resting or activated T cells (L. Chen *et al.*, unpublished data). Thus, our results suggest that blockade of the MDC B7-H1 pathway facilitates a signal(s) from T cells that modulates MDC cytokine secretion and subsequently increases the T-cell stimulatory capacity of MDCs.

### Blockade of MDC-B7-H1 improves T cell antitumor immunity

To determine whether B7-H1 expression on MDCs affected activation of tumor-associated T cells, we stimulated T cells isolated from ascites of human ovarian carcinoma with autologous lipopolysaccharide-activated tumor MDCs loaded with irradiated autologous tumor cells as the source of tumor antigens. Tumor MDCs induced T-cell IFN-γ production. When B7-H1 was blocked, however, significantly more T-cell IFN-γ was produced ($P < 0.001$; Fig. 4*a*). No significant T-cell IL-10 (<40 pg/ml) was induced by tumor MDCs treated with monoclonal antibody against B7-H1, in contrast to the significant T-cell IL-10 expression induced by tumor MDCs without such treatment ($P < 0.05$; Fig. 2).

To test their antitumor effect, these MDC-activated T cells were then injected into NOD-SCID mice bearing the corresponding established tumors. Mice without T-cell transfusion and mice treated with MDC-activated tumor T cells in the presence of control monoclonal antibody (non-conditioned T cells) showed progressive tumor growth, although tumor T cells partially reduced the tumor volume. Mice treated with MDC-activated tumor T cells in the presence of a monoclonal antibody against B7-H1 (conditioned T cells) showed no tumor growth at each time point through day 16 after T-cell transfusion, at which time tumors resumed slow growth (Fig. 4*b*).

We isolated tumors from mice 12 d after T-cell transfusion and analyzed the infiltration of T cells. The number of human IFN-γ-

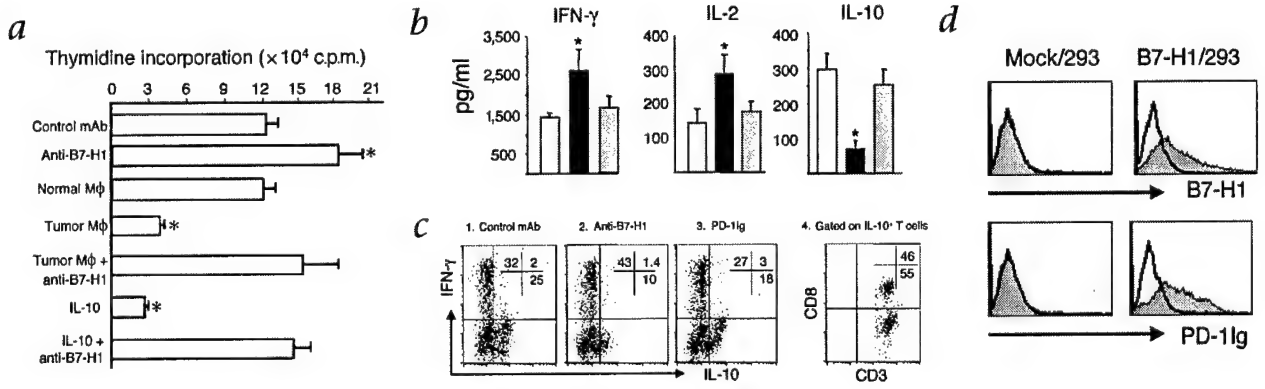



**Fig. 2** Function of MDC-associated B7-H1. ***a***, Proliferation of T cells induced by blood MDCs cultured under the indicated conditions, as measured by thymidine incorporation. *, $P < 0.01$ as compared with MDCs plus control monoclonal antibodies (mAb). $n = 5$. MΦ, macrophage; anti-B7-H1, antibody against B7-H1. ***b*** and ***c***, T-cell cytokines were detected by ELISA (*b*) and intracellular staining (*c*) on day 6 after the last stimulation with MDC. Panels 1–3 are gated on $CD3^+$ T cells; panel 4 is gated on $IL\text{-}10^+$ cells. Numbers in quadrants represent percentage of positive cells. □, control monoclonal antibody; ■, antibody against B7-H1; ▒, Pd-1Ig. $n = 5$. *, $P < 0.05$. ***d***, 293 cells were transfected with empty pcDNA3 vector (Mock/293) or B7-H1-pcDNA3 vector (B7-H1/293) for 48 h and stained with control mAb (top, bold line) or antibody against B7-H1 (top, regular line). The B7-H1–transfected 293 cells were stained with control human IgG (bottom, bold line) and human PD-1Ig (bottom, regular line). The binding of antibodies or immunoglobulin proteins was detected using FITC-conjugated antibodies against mouse or human IgG.

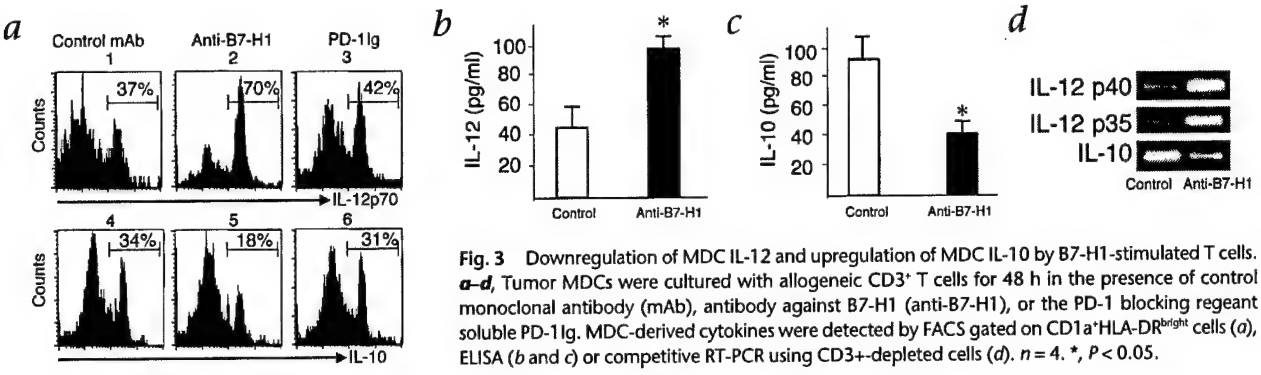
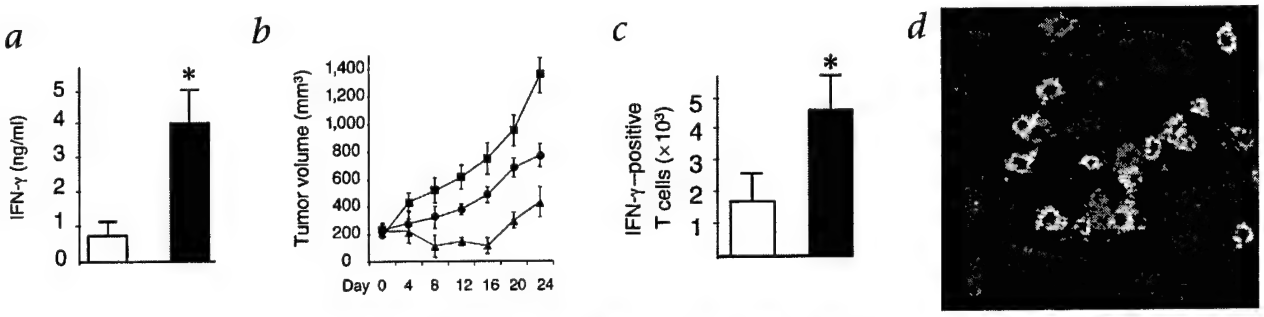
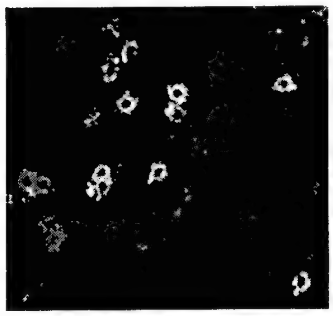



**Fig. 3** Downregulation of MDC IL-12 and upregulation of MDC IL-10 by B7-H1-stimulated T cells. ***a–d***, Tumor MDCs were cultured with allogeneic $CD3^+$ T cells for 48 h in the presence of control monoclonal antibody (mAb), antibody against B7-H1 (anti-B7-H1), or the PD-1 blocking regeant soluble PD-1Ig. MDC-derived cytokines were detected by FACS gated on $CD1a^+HLA\text{-}DR^{bright}$ cells (*a*), ELISA (*b* and *c*) or competitive RT-PCR using CD3+-depleted cells (*d*). $n = 4$. *, $P < 0.05$.

positive T cells in tumors was significantly higher in the group treated with conditioned T cells as compared with mice treated with non-conditioned T cells ($P < 0.001$; Fig. 4*c*). We did not, however, detect IL-10-positive T cells in the tumor mass by intracellular staining. Confocal microscopy analysis showed similar numbers of human $CD8^+CD3^+$ T cells (352 ± 62 cells in 10 high-powered fields compared with 371 ± 82) and $CD8^-CD3^+$ T cells (132 ± 37 compared with 148 ± 55) migrating into tumors from mice treated with conditioned or non-conditioned T cells, respectively (Fig. 4*d* and *e*). We did not observe a significant difference in apoptotic human tumor-infiltrating T cells in mice that received the conditioned T cells (Fig. 4*f*) as compared with mice that received the non-conditioned T cells (12 ± 5 cells in ten high-powered fields compared with 13 ± 6 $CD3^+$ apoptotic T cells), suggesting that T cells conditioned with monoclonal antibody against B7-H1 are not more resistant to apoptosis. These



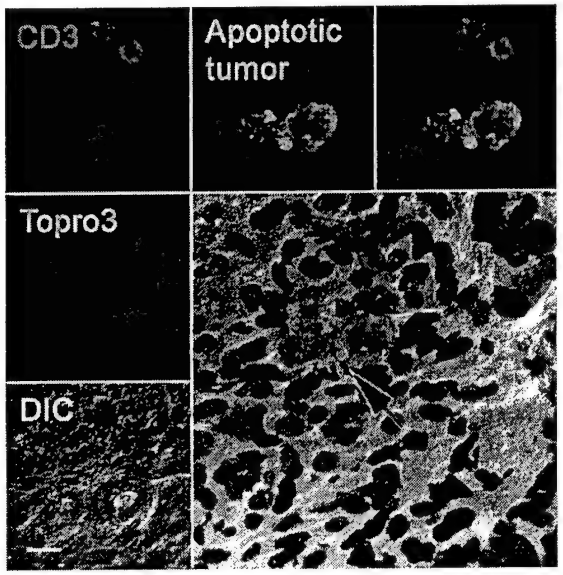




**Fig. 4** Tumor MDC–associated B7-H1 inhibits tumor immunity. ***a***, Blocking tumor MDC–associated B7-H1 significantly enhanced tumor T-cell IFN-γ induction by ELISA (□, control monoclonal antibody; ■, antibody against B7-H1). *, $P < 0.001$. T-cell IFN-γ was <0.05 ng/ml using tumor MDCs loaded with apoptotic U937 cells or MDCs without apoptotic cell loading. Error bars represent mean ± s.e.m. of triplicate determinations. Shown is 1 representative experiment of 4. ***b***, Blocking tumor MDC–associated B7-H1 enhances tumor growth inhibition in NOD-SCID mice bearing established human ovarian carcinoma tumors. Tumor T cells were stimulated twice with autologous tumor MDCs plus control monoclonal antibody (●; $n = 5$; referred to as 'non-conditioned' in text) or monoclonal antibody against B7-H1 (▲; $n = 5$; referred to as 'conditioned' in text). A group of tumor-bearing mice did not receive T cells (■; $n = 7$). Mean ± s.d. of tumor volumes is shown. The T-cell injection day was counted as day 0. $P < 0.05$ at all time points from days 8 to 24, as compared with either control group. ***c***, Tumor-infiltrating IFN-γ–positive T cells were quantified by FACS, expressed as cells per tumor (□, control monoclonal antibody; ■, antibody against B7-H1). *, $P < 0.001$; $n = 5$. ***d*** and ***e***, Tumor-infiltrating T cells were stained with mouse antibodies against human CD3 (red) and CD8 (green). Similar numbers of tumor-infiltrating T cells were observed in mice treated with conditioned (*d*) and non-conditioned (*e*) T cells. ***f***, Images for individual channels are shown on the left and top; a larger merged image containing all channels is shown on the right. Arrow indicates an apoptotic T cell (yellowish). Red, $CD3^+$ T cells; green, apoptotic cells; grayscale, differential interference contrast (DIC); blue, Topro3-stained nuclei. Scale bar, 10 μm.

data indicate that blockade of tumor MDC–associated B7-H1 facilitates infiltration of IFN-γ–secreting CD8⁺ T cells that correlate with protection against tumor growth.

## Discussion

To evade host immunity, tumors use numerous strategies to hinder normal MDC function, differentiation and trafficking. For example, the tumor-associated cytokines IL-6, macrophage colony-stimulating factor and VEGF inhibit MDC differentiation and maturation[9,21], preventing activation of potentially protective antitumor immunity. We have shown that human tumor environmental factors upregulate B7-H1 on differentiated MDCs, which subsequently leads to impaired T-cell antitumor immunity. This new finding is distinct from our previous work showing that human cancer-associated B7-H1 promotes apoptosis of effector T cells, and points to a new mechanism by which human cancers evade immunity.

Differentiated MDCs also enter the tumor microenvironment and may be phenotypically or functionally altered as a result. In this regard, we showed that MDCs in tumor-draining lymph nodes, and those derived from tumor CD14⁺ cells, expressed significantly more B7-H1 than blood MDCs, suggesting that tumor microenvironmental factors induce MDC B7-H1 expression. We also identified tumor macrophages and tumor epithelial cells as sources of IL-10 and VEGF, respectively; these cytokines are involved, at least in part, in induction of MDC B7-H1. Nevertheless, the intensity of MDC B7-H1 expression induced by recombinant IL-10 and VEGF or purified tumor macrophages and tumor cells was lower than that on MDCs in tumor-draining lymph nodes and tumors, suggesting involvement of additional factors *in vivo* in B7-H1 induction.

The T-cell CD40 ligand (CD40L) is a critical signal for activating MDCs to induce antigen-specific T-cell responses[22–24]. Molecular pathways involved in negative regulation of dendritic cell function remain largely unknown, however. CD8⁺CD28⁻ T cells provide as-yet-unidentified signals for upregulating dendritic-cell immunoglobulin-like transcript receptors (ILT)-3 and ILT-4 to induce T-cell tolerance[25]. Normal MDCs express low levels of B7-H1, and receptors for B7-H1 are not normally found on naive T cells[10,12]. Tumor MDCs, however, express high levels of B7-H1, which could engage T cells, leading to downregulation of MDC IL-12 and upregulation of IL-10. Because MDC IL-12 is crucial for establishing tumor-specific immunity and Th1 polarization[26], and because IL-10 inhibits tumor-specific immunity in ovarian carcinomas[8], MDC-associated B7-H1 signals could determine the nature of subsequent T-cell activation. In support of this concept, we found that blockade of tumor MDC–associated B7-H1 decreases T-cell IL-10 expression, increases T cell IFN-γ production and improves clearance of tumor in xenotransplanted mice. This clearance is associated with tumor infiltration by IFN-γ–secreting T cells. We propose that B7-H1 blockade improves the effector function of tumor-infiltrating T cells, based on our *in vivo* observations. Protection from T-cell apoptosis does not seem to be a mechanism by which B7-H1 blockade improves tumor infiltration with IFN-γ–secreting T cells.

PD-1Ig had minimal effects on MDC-induced T-cell cytokine secretion. In addition, although the monoclonal antibody 5H1 blocked inhibitory effects of tumor-associated MDCs, 5H1 does not block the binding between B7-H1 and PD-1[27] (L. Chen *et al.*). We previously reported that B7-H1Ig, a soluble fusion protein prepared by fusing the extracellular domain of human B7-H1 to the CH2-CH3 domain of mouse immunoglobulin G2a in the expression plasmid pmIgV, could bind a PD-1–negative human T-cell clone, and that the apoptotic effect of tumor-associated B7-H1 on some human T-cell clones could be blocked by 5H1 but not by PD-1Ig[12]. Taken together with our recent observation that B7-H1Ig can co-stimulate growth and cytokine secretion of T cells isolated from PD-1–deficient mice[28] (L. Chen *et al.*), these findings support the existence of receptor(s) in addition to PD-1.

Our results suggest that blocking B7-H1 could be therapeutic in ovarian carcinoma. This notion is directly supported by our experiments showing increased regression of xenografted ovarian cancers after adoptive transfer of conditioned T cells in the presence of B7-H1 blockade. A single injection of T cells activated with MDCs in the presence of B7-H1 blockade significantly slowed tumor growth. Repeated treatments would probably mediate additional benefits. Reduced growth correlated with tumor infiltration by IFN-γ–positive CD8⁺ T cells, suggesting a plausible mechanism of action. A similar mechanism may also operate in other cancers as the production of IL-10 and VEGF in the tumor microenvironment has been reported in many other cancers[29–32].

Many tumors, including the majority of ovarian carcinomas, express B7-H1. Tumor B7-H1 induces T-cell apoptosis and is likely to contribute to immunopathology[12]. Thus, blocking B7-H1 signals may be useful to treat certain cancers not only by inhibiting immunosuppression from B7-H1–expressing MDCs, but also by preventing tumor-mediated T-cell apoptosis through tumor B7-H1.

## Methods

**Human subjects and clinical samples.** Subjects gave written, informed consent. The study was approved by the Institutional Review Board of the Tulane Medical School. Malignant ascites and tumor-draining lymph nodes were from previously untreated ovarian carcinoma patients. Control lymph nodes were from subjects without tumors. Ascites cells (macrophages, tumor cells and T cells) were prepared as described[8]. Normal macrophages were produced as described[33].

**MDCs.** Normal MDCs and tumor MDCs were differentiated *in vitro* from peripheral blood monocytes and malignant ascites macrophages, respectively[33]. LN-MDCs were prepared by mechanical disruption of freshly obtained lymph nodes draining the retroperitoneum of patients. Lineage-positive cells were depleted as described[8] and LN-MDCs were determined by flow cytometry gating on lineage-negative CD11c⁺HLA-DR$^{bright}$ cells. Blood and tumor MDCs on day 6 of culture were used for *in vitro* experiments. MDCs were activated by culture for 2 additional days with *Escherischia coli* lipopolysaccharide (1 μg/ml, Sigma, St. Louis, Missouri) for *in vivo* experiments.

**MDC phenotype and cytokines.** MDC phenotype was determined by flow cytometry analysis using specific monoclonal antibodies (all from BD PharMingen, San Diego, California). MDC cytokines were detected by intracellular staining (PharMingen; gated on CD1a⁺HLA-DR$^{bright}$ cells), ELISA (R&D Systems, Minneapolis, Minnesota) and competitive RT-PCR[34]. MDCs were purified from T-cell co-culture using CD3 magnetic bead depletion (Miltenyi, Auburn, California).

**Transwell experiments.** Blood MDCs ($5 \times 10^5$ cells/ml) were added to the lower chambers of 0.4-μm pore size Transwells (Costar, Cambridge, Massachusetts). Tumor macrophages ($10^6$ cells/ml) or primary ovarian carcinoma cells ($10^6$ cells/ml) were added to the upper chamber. Recombinant human IL-10 (1–20 ng/ml), monoclonal antibody against human IL-10 (500 ng/ml), recombinant human VEGF (1–20 ng/ml) or VEGFR Fc (3 ng/ml; all from R&D Systems) were added as indicated. After 48 h, B7-H1 expression on blood MDCs was determined by FACS, and blood MDCs were used to stimulate allogeneic T-cell responses.

**Allogeneic mixed lymphocyte reaction.** Allogeneic CD3⁺ T cells were stimulated twice, 1 week apart, with MDCs. Where indicated, MDCs were pre-in-

cubated for 1 h with monoclonal antibody against B7-H1 (5H1; 500 ng/ml)[12] and T cells were pre-incubated for 1 h with PD-1Ig (30 μg/ml)[12] as indicated. T-cell proliferation and cytokine production were assessed on day 6 after the second stimulation.

**Activation of tumor-associated T cells.** Autologous tumor MDCs were incubated with irradiated apoptotic ovarian carcinoma cells or apoptotic U937 cells (American Type Culture Collection, Manassas, Virginia) in a 1:10 ratio as previously described[8,35] for 2 h, activated with lipopolysaccharide (1 μg/ml) for 48 h and co-cultured with autologous tumor ascites T cells. After 1 week, a second identical stimulation was performed. Monoclonal antibodies against human B7-H1 and human IgG1 (1 mg/ml, BD PharMingen) were added as indicated. The resulting cell suspension was comprised of 25 ± 8% $CD4^+$ T cells and 67 ± 12% $CD8^+$ T cells. These T cells were injected into NOD-SCID mice bearing previously established human ovarian carcinomas autologous to the T cells.

**Immunohistochemistry and confocal analysis.** Immunohistochemistry was performed on 8-μm cryosections of acetone-fixed tissues. Tumor tissues were incubated for 1 h at room temperature with rabbit polyclonal antibody against human CD3 (1:10; Dako, Carpinteria, California) and biotin-labeled mouse IgG1 against human CD8 (clone DK25; 5 μg/ml; BD PharMingen) followed by Alexa 568-conjugated goat antibody against rabbit IgG (1:1,000; Molecular Probes, Eugene, Oregon) and streptavidin-labeled Alexa 488 (Molecular Probes) for 30 min. Apoptotic cells were detected with the ApopTag Kit (Intergen, Norcross, Georgia). The number of positive cells was expressed as the mean ± s.d. in 10 different ×200 fields using confocal microscopy with a Leica TCS-NT SP equipped with argon, krypton, and helium/neon lasers.

**NOD-SCID mouse model.** The study was approved by the Institutional Animal Care and Use Committee of the Tulane Medical School. Female NOD.CB17-SCID mice (6–8 weeks old; Jackson Laboratory, Bar Harbor, Maine) were used as previously described[36]. Primary ovarian tumor cells ($2 \times 10^7$) in 200 μl of buffered saline were injected into dorsal subcutaneous tissues[37]. Tumor size was measured twice weekly using calipers fitted with a Vernier scale. Tumor volume was calculated based on 3 perpendicular measurements[37]. Tumor-associated T cells were conditioned with MDCs as described above. The activated tumor-associated T cells ($5 \times 10^6$) were injected into the peritoneum in 100 μl buffered saline on day 12 after human tumor inoculation. In some cases, tumor tissues were extracted from mice 12 d after T-cell infusion and mechanically disrupted into a single-cell suspension. Cells were analyzed by FACS for human IFN-γ expression by intracellular staining, gating on human $CD3^+$ cells. Human T-cell tumor infiltration and apoptosis were further assessed using immunohistochemistry and analyzed using confocal microscopy.

**Statistical analysis.** Differences in cell surface molecule expression were determined by $\chi^2$-test, and in other variables by unpaired *t*-tests, with $P < 0.05$ considered significant.

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*Competing interests statement*

*The authors declare that they have no competing financial interests.*

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GR and 2 affected dogs of other breeds. An unaffected GR (with a diagnosis of reactive lymphoid hyperplasia) was included as a control. Our results show that inactivation of Rb was present in all tumors, and occurred through various mechanisms including deletion of p16, overexpression of cyclin D, or hyperphosphorylation of Rb. Intriguingly, the patterns of CDK4 (or CDK6)-dependent Rb phosphorylation appeared to differ between B cell and T cell lymphomas. To confirm this, we developed a means to examine site-specific Rb phosphorylation using immunohistology, and evaluated the patterns of Rb phosphorylation retrospectively in 17 cases. A "common" lesion was not identifiable in related or unrelated dogs in this sample set, suggesting that the tumors were sporadic, or that possible heritable predisposing traits for lymphoma are independent of this pathway. Nevertheless, the results and materials derived from these investigations offer a unique source to explore important questions of cancer susceptibility. Supported by AKC CHF 1626, 2025, 2254.

**161.4**

**Epstein Barr Virus Can Protect Latently Infected B Cell Lymphomas From FasL- and TRAIL-Mediated Apoptosis**

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Epstein Barr virus (EBV)-associated B cell lymphomas remain a significant problem in immunocompromised individuals, for which alterations in T cell effector pathways may provide growth advantages to virally-infected cells. We sought to determine whether EBV itself could modulate apoptosis specifically through death receptors in latently infected B cells.

Unlike BJAB parent lymphoma cells, BJAB cells latently infected with B95.8 EBV (BJAB_B95) are completely resistant to apoptosis induced through the FasL and TRAIL pathways. Cell surface expression of Fas is moderately decreased in BJAB_B95 compared with BJAB, whereas expression of the TRAIL receptors is unaltered. Although death inducing signaling complex (DISC) formation in BJAB_B95 is intact upon death receptor engagement, impaired processing and activation of caspases 8 and 3 is evident. Furthermore, concomitant treatment with cycloheximide restores sensitivity to both apoptotic stimuli, indicating that translation of inhibitory protein(s) driven by latent EBV infection confers resistance to death receptor apoptosis in EBV-infected BJAB cells.

In summation, we report novel evidence that EBV itself can protect latently infected B cells from death receptor apoptosis. Determining EBV-influenced mechanisms of resistance to FasL- or TRAIL-induced apoptosis may aid in characterization and treatment of EBV-associated malignancies.

**161.5**

**Tumor-associated plasmacytoid dendritic cells enhance, whereas myeloid dendritic cells inhibit tumor angiogenesis**

**weiping zou**[1], Xavier Alvarez[2], Pui Cheng[3], Andrew Lackner[2], Peter Carmeliet[4], Tyler Curiel[1]. [1]medicine, Tulane Medical school, 1430 Tulane avenue, New orleans, LA 70112, [2]Primate Center, Tulane Medical school, New orleans, LA, [3]Gynecoclogy, Tulane Medical school, New orleans, LA, [4]Center for Transgene Technology & Gene Therapy, Leuven, Belgium

Ovarian carcinomas have a poor prognosis, often associated with multifocal intraperitoneal dissemination accompanied by intense neovascularization. To examine tumor angiogenesis in the tumor microenvironment, we studied ascites of patients with untreated ovarian carcinoma. We observed high levels of plasmacytoid dendritic cells (PDC) and stromal derived factor (SDF)-1. Tumor derived SDF-1 attracts PDC into the tumor environment. We now show that tumor PDC independently induce angiogenesis in vitro and in vivo (Matrigel in mice) that is synergistically augmented by tumor SDF-1. Tumor, but not blood PDC spontaneously secreted IL-8 and TNF-alpha that may contribute to this process. By contrast, myeloid dendritic cells (MDC) were absent from malignant ascites. In vitro differentiated MDC suppressed in vitro angiogenesis, suggesting an effect opposite from PDC. Although DC have long been known to affect tumor immunity, these data now also implicate them in tumor neoangiogenesis. Further, tumors appear to manipulate DC distribution and function to favor tumor neovascularization. Tumor SDF-1 and its target PDC form a novel angiogenic pathway. Targeting this pathway may be applicable to development of anti-angiogeneic therapies.

**161.6**

**EBV induces Ig class switch DNA recombination and enhances malignant B cell growth and survival by up-regulating BAFF and APRIL through LMP1**

**Andrea Cerutti**[1], Nancy Raab-Traub[2], Amy Chadburn[1], Elaine J. Schattner[3], Daniel K. Knowles[1], Paolo Casali[1], Bing He[1]. [1]Pathology, Weill Medical College of Cornell University, 1300 York Avenue, New York, NY 10021, [2]University of North Carolina, Chapel Hill, NC, [3]Medicine, Weill Medical College of Cornell University, New York, NY

BAFF and APRIL are dendritic cell-derived molecules that favor B cell survival and proliferation. The role of BAFF and APRIL in B cell neoplasias, including non-Hodgkin's lymphoma (NHL), remains unknown. Here we show that the lymphomagenic virus EBV induces BAFF and APRIL in B cells through an LMP1-mediated NF-κB-dependent mechanism. By engaging TACI, BCMA and BAFF-R, autocrine BAFF and APRIL favor the accumulation of neoplastic B cells and trigger IgH class switch DNA recombination (CSR), a process critically involved in the pathogenesis of NHL-associated chromosomal translocations. These effects are associated with up-regulation of activation-induced cytidine deaminase (AID), a critical component of the IgH CSR machinery, up-regulation of c-Myc, an inducer of cell proliferation, up-regulation of pro-survival Bcl-2 and Bcl-xL, down-regulation of pro-apoptotic Bax, and down-regulation of p53, an inducer of cell cycle arrest. Although at lower levels, BAFF and APRIL are also expressed by EBV- NHLs. This might result from external stimuli, including CD40L or antigen, or overexpression of certain transcription factors, including NF-κB. By showing that soluble TACI and BCMA decoy receptors interfere with the growth of neoplastic B cells, our findings suggest that BAFF and APRIL play an important role in lymphomagenesis.

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**161.7**

**Isoforms of human Terminal deoxynucleotidyl Transferase are expressed in developing lymphocytes and leukemias**

**Ryan Scott Schulz**, To-Ha Thai, John F Kearney. Microbiology, University of Alabama at Birmingham, 378 WTI 1824 6th Ave. S, Birmingham, AL 35294

Mouse terminal deoxynucleotidyl transferase (TdT) exists in two splice forms, TdTS and TdTL, which are identical except for a twenty amino acid insertion between exons X and XI in the TdTL isoform. This insertion causes TdTL to function as a 3'-5' exonuclease as opposed to the 5'-3' nontemplated polymerase activity of TdTS. A search of the human genome revealed that huTdT can potentially exist as three splice variants: TdTS, TdTL2, and TdTL1. Real time PCR and conventional PCR developed for all three splice variants of huTdT in normal, developing lymphocytes, cell lines, and leukemias indicate that all three splice variants can be expressed at the RNA level. The functions of the human long isoforms are unknown and are currently under study. TdT is an important enzyme in lymphocyte development and is also expressed in some human leukemias. The functional relevance of TdT isoform expression in normal and leukemia cells is unknown. Unlike previous studies examining the relationship between TdT and leukemias, we are able to quantify the level, in ALLs and AMLs, of RNA expression of each TdT isoform using real time PCR. We have shown that TdTS is expressed in most lymphoid leukemias, but that the expression of TdTL2 is highly variable among different patients. TdTL1 does not appear to be expressed in these samples. The level of expression of each splice variant may serve as useful prognostic and diagnostic tools for the treatment of leukemia.

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| Zou, Weiping, M.D., Ph.D. | Assistant Professor of Medicine |
|---|---|

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| Tongji Medical School, Wuhan | M.D. | 1990 | Medicine |
| Paris University, France | Ph.D. | 1997 | Immunology |
| Baylor Institute for Immunol. Res., Dallas | Post-doc | 2001 | Immunology |

**A. Positions and Honors.**

**Employment**

September 2001-present. Assistant Professor of Medicine at Tulane University, New Orleans, LA
Research on dendritic cells and tumor immunopathology

1999-2001 Post-doctoral fellow at Baylor Institute for Immunology Research, Dallas, TX
Research on dendritic cell biology in human cancer

1994-1998 Ph.D student at Institut Paris-Sud sur les Cytokines, INSERM U131, France
Research on chemokine/cytokine biology in HIV/SIV-infection

1993-1994 Visiting scientist at Institut Pasteur; Institut Paris-Sud sur les Cytokines, France
Developing a technique to quantify cytokine mRNA.
Research on autoimmune diseases (SLE and Wegener's disease)

1990-1993 Clinical fellow at Tongji Medical School, China
Research on Pharmacokinetics and Immunopharmacology
Physician in Infectious Diseases, Lecturer on Immunopharmacology

1982-1990 Medical student at Tongji Medical School, China
Research on pharmacology

**B. Publications**

Kryczek I, Cheng P, Mottram P, Alvarez X, Moons L, Evdemon-Hogan M, Wei S, Zou L, Machelon V, Emilie D, Carmeliet P, Curiel TJ, Lackner A, **Zou W**. CXCL-12 and VEGF synergistically induce neoangiogenesis in human ovarian cancers. **Submitted**

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**Zou W,** Borvak J, Wei S, Marches F, Isaeva T, Curiel DT, Crippin J, Gordon AN, Curiel TJ. Reciprocal regulation of human plasmacytoid dendritic cells and monocytes during viral infection. **Euro J Immunol 2001, 31:3833-3839. (Corresponding author)**

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